

June 16, 2003

Christine Todd Whitman, Administrator
U.S. Environmental Protection Agency
Ariel Rios Building
Room 3000, #1101-A
1200 Pennsylvania Ave., N.W.
Washington, DC 20460

Subject: Comments on the HPV Test Plan for 3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde (HMPCC)

Dear Administrator Whitman:

The following comments on the Flavor and Fragrance Consortium's High Production Volume Challenge test plan for the chemical 3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde, referred to as HMPCC, are submitted on behalf of the Physicians Committee for Responsible Medicine, People for the Ethical Treatment of Animals, the Humane Society of the United States, the Doris Day Animal League, and Earth Island Institute. These health, animal protection, and environmental organizations have a combined membership of more than ten million Americans.

The Flavor and Fragrance High Production Volume Consortium, on behalf of the member companies of the Alicyclic Aldehyde Consortium, submitted its test plan on February 25, 2003 and produces the chemical HMPCC (CAS No. 31906-04-4), a colorless, viscous liquid with an aroma reminiscent of lily of the valley. HMPCC is composed of a 70:30 mixture of two isomers and this mixture is the predominant product of commerce where it is found as a fixative in soap, cosmetics, and perfumes at low concentrations. HMPCC has not been reported to occur naturally but shares structural similarities with the naturally occurring chemical, 7-hydroxycitronellal. This consortium proposes to conduct an acute aquatic toxicity study in fish, OECD 203, and a reproductive/developmental study in rats, OECD 421. If both of these tests are conducted, 715-795 animals will have been killed.

This test plan utilizes existing studies on HMPCC as well as incorporating data from structurally related chemicals such as hydroxycitronellal, hydroxycitronellol, and perilla aldehyde to fill in data gaps for physicochemical properties, environmental fate, and toxicity endpoints. This is a scientifically valid analysis and adequate for a screening level program such as HPV. In addition, this approach is consistent with the EPA's

stated goal of maximizing the use of existing data in order to limit additional animal testing.

At this time, however, we question the Flavor and Fragrance Consortium's assessment that an acute toxicity study in fish (OECD 203) and a combined reproductive/developmental toxicity study (OECD 421) are needed to meet the requirements of the HPV program. These tests were proposed because no data were available to meet these SIDS endpoints.

First, the n-Octanol/water partition coefficients ($\log K_{O/W}$) and water solubility values of HMPCC should be determined before proposing to conduct an acute aquatic toxicity study which will result in the death of 40-120 fish. These factors may affect the behavior of this chemical and are likely to affect its aquatic toxicity. Furthermore, MSDS information on hydroxycitronellal, a structural relative of HMPCC, indicates that it is insoluble in water. This may also be true of HMPCC. However, determination of $\log K_{O/W}$ and water solubility values will make this clear and therefore, must be determined prior to proposing any study that will result in the death and suffering of many animals.

In addition, this test plan proposes to conduct a reproductive/developmental toxicity test, which will result in the death of at least 675 rats, without adequately interpreting data from existing studies. In a subchronic inhalation study of hydroxycitronellal with rats and hamsters, no toxicologically significant effects on animal survival, behavior, body weights or weight gains, or organ weights (including **uterus, testes and ovaries**) were reported and no gross pathological (**uterus, testes, and ovaries**) or histopathological (**uterus and testes**) findings were observed in either species (Fukayama *et al.*, 1999). In another repeat-dose study, this time using perilla aldehyde via oral gavage in rats and dogs, no alterations in organs and tissues, including **ovaries and gonads**, were revealed after histopathological examination (National Cancer Institute, 1996).

In this latter study, we question if this route, oral gavage, is accurately reported for dogs. Gavage is not a common route of exposure in dogs, as opposed to capsules. The information in this test plan is also confusing in that while rats and dogs were mentioned in the test plan (p. 18), only rats were mentioned in the robust summaries (p. 41, section 4.3). These discrepancies in route of exposure and species tested need to be clarified. Furthermore, upon searching for this reference, we were unable to locate this article in the *Journal of Cellular Biochemistry*. Nonetheless, due to similarities between hydroxycitronellal, perilla aldehyde, and HMPCC, data from the aforementioned studies in rats and hamsters should be used to extrapolate to HMPCC to fulfill the required SIDS endpoint for reproductive toxicity for this chemical. In doing so, a thoughtful, qualitative analysis of HMPCC will have been carried out, rather than a rote checklist approach to toxicology. There is no indication based on repeat dose studies in rats and hamsters that this compound poses a reproductive hazard.

If the Flavor and Fragrance Consortium wishes to investigate the potential developmental effects of HMPCC, we strongly urge it to consider an *in vitro* method, in order to spare large numbers of animals. The rodent embryonic stem cell test, an *in vitro*

embryotoxicity test method, has recently been validated by the European Centre for the Validation of Alternative Methods, and the Centre's Scientific Advisory Committee has concluded that this test is ready to be considered for regulatory purposes (Genschow, 2002). If a positive result is found in the embryonic stem cell test, HMPCC should be treated as a development toxicant/teratogen, and no further testing should then be carried out within this screening-level program. Although we have written to the EPA repeatedly concerning the inclusion of the embryonic stem cell test in the HPV Program, with correspondence dating back more than six months, we have received no reply. We urge the Flavor and Fragrance Consortium to correspond directly with the EPA on the incorporation of this validated non-animal test.

I look forward to a prompt and favorable response to our concerns. I may be reached at 202-686-2210, ext. 327, or via e-mail at meven@pcrm.org.

Sincerely,

Megha Even, M.S.
Research Analyst

Chad B. Sandusky, Ph.D.
Director of Research

References:

Fukayama MY, Easterday OD, Serafino PA, Renskers KJ, North-Root H, Schrankel KR. Subchronic inhalation studies of complex fragrance mixtures in rats and hamsters. *Toxicol. Letters* 111: 175-187, 1999.

Genschow E., *et al.* The ECVAM international validation study on *in vitro* embryotoxicity tests: Results of the definitive phase and evaluation of prediction models. *Altern. Lab. Anim.* 30: 151-76, 2002.

National Cancer Institute, Division of Cancer Prevention and Control, Chemoprevention Branch and Agent Development Committee. Clinical development plan: l-perillyl alcohol. *Journal of Cellular Biochemistry* 265: 137-148, 1996.